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Development of aqueous two-phase systems based on deep eutectic solvents for continuous protein extraction in a microextractor

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Introduction

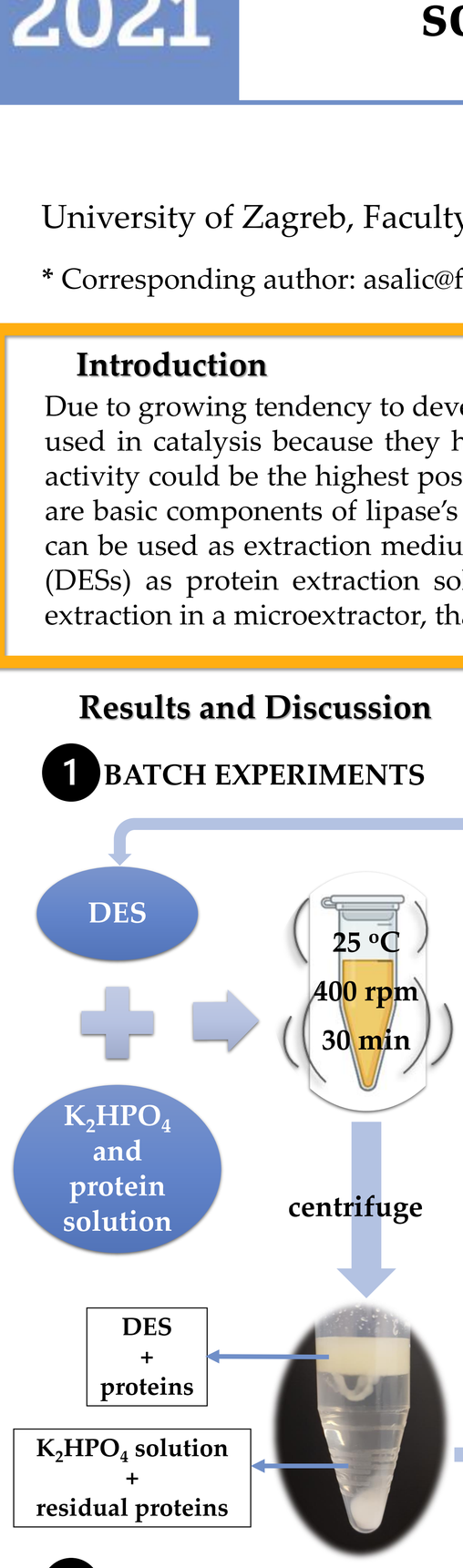
Due to growing tendency to develop processes that are more eco-friendly, the use of enzymes as catalysts is gaining more and more attention. Lipases are enzymes very often used in catalysis because they have a very wide specificity and maintain high activity in mild conditions. These trends require an efficient lipase purification so that its activity could be the highest possible. Conventional enzyme purification techniques are very expensive, time consuming, and, most importantly, complicated. Since proteins are basic components of lipase's structure, aqueous two-phase protein extraction is considered as a promising and flexible alternative purification method. Various solvents can be used as extraction mediums and so, in accordance with the principles of green chemistry, the use of biodegradable, non-toxic, and recyclable deep eutectic solvents (DESs) as protein extraction solvents is intensively investigated. Therefore, aqueous two-phase system (ATPSs) based on natural DES and its application for protein extraction in a microextractor, that ensures higher efficiencies due to microchannel geometry and a continuous process, were investigated in this research.

Results and Discussion

1 BATCH EXPERIMENTS

Table 1. Synthesized DESs and their properties

DES	abbreviation	component molar ratio	water content, %	ρ, g/mL	pH	η (25°C), mPas	ν (25°C), mm²/s
cholin-chloride: urea	ChU	1: 2	5	1.185	9.321	30.686	25.895
cholin-chloride: glycerol	ChGly	1: 2	-	1.150	5.324	53.099	46.173
cholin-chloride: ethylene glycol	ChEG	1: 2	-	1.135	6.981	15.536	13.688
cholin-chloride: glucose	ChGlc	1: 1	25	1.265	5.691	38.785	30.660
betain: glycerol	BGly	1: 2	-	1.180	9.255	212.193	179.825
betain: urea	BU	1: 3	40	1.190	9.254	3.472	2.918



$$E = \frac{\gamma_{P,\text{upper phase}} \cdot V_{\text{upper phase}}}{\gamma_{P,\text{upper phase}} \cdot V_{\text{upper phase}} + \gamma_{P,\text{bottom phase}} \cdot V_{\text{bottom phase}}}$$

Bradford protein assay

$\gamma_{P,\text{upper phase}}$, $\gamma_{P,\text{bottom phase}}$

Optimal two-phase system features:
✓ DES BU
✓ $\gamma_{K_2HPO_4}$ = 0.7 g/mL

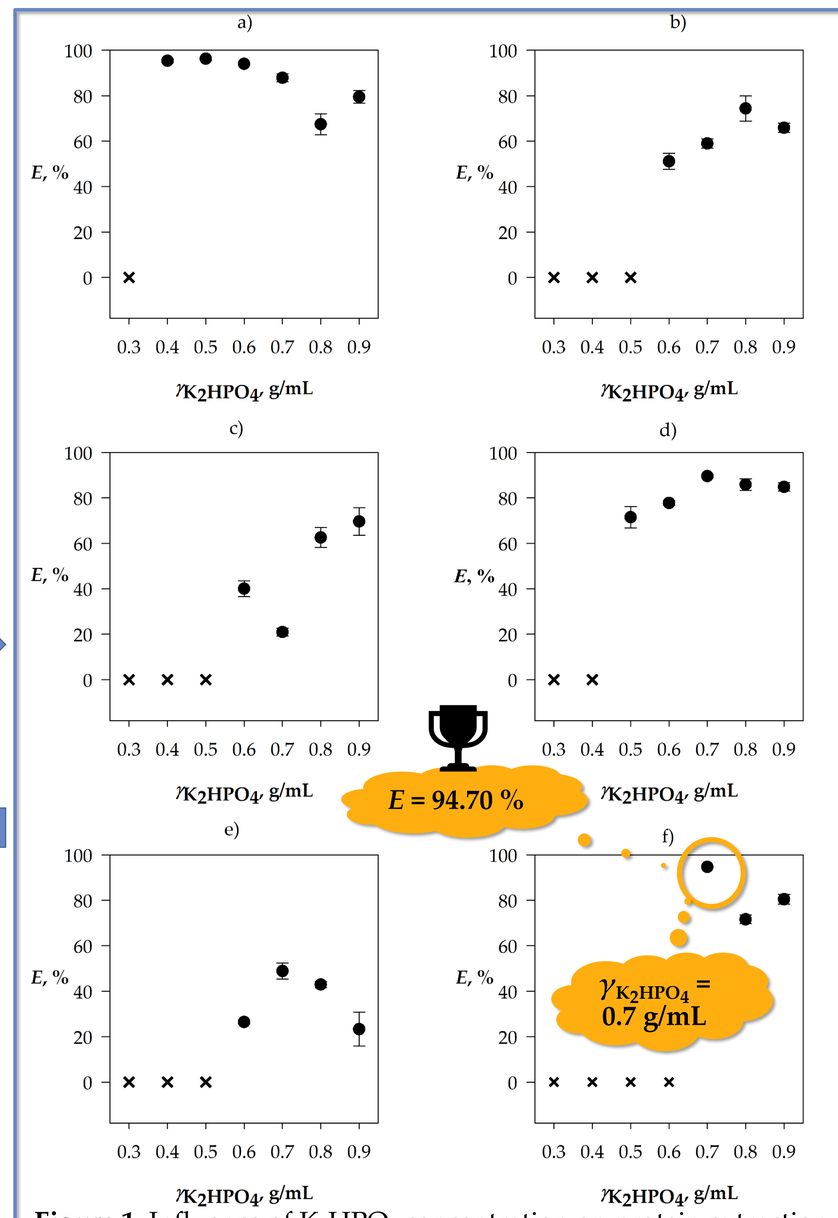


Figure 1. Influence of K_2HPO_4 concentration on protein extraction efficiency in ATPS based on (a) ChU, (b) BGly, (c) ChGly, (d) ChGlc, (e) ChEG and (f) BU (**x** no phase formation, • two-phase system)

2 EXPERIMENTS IN A MICROEXTRACTOR

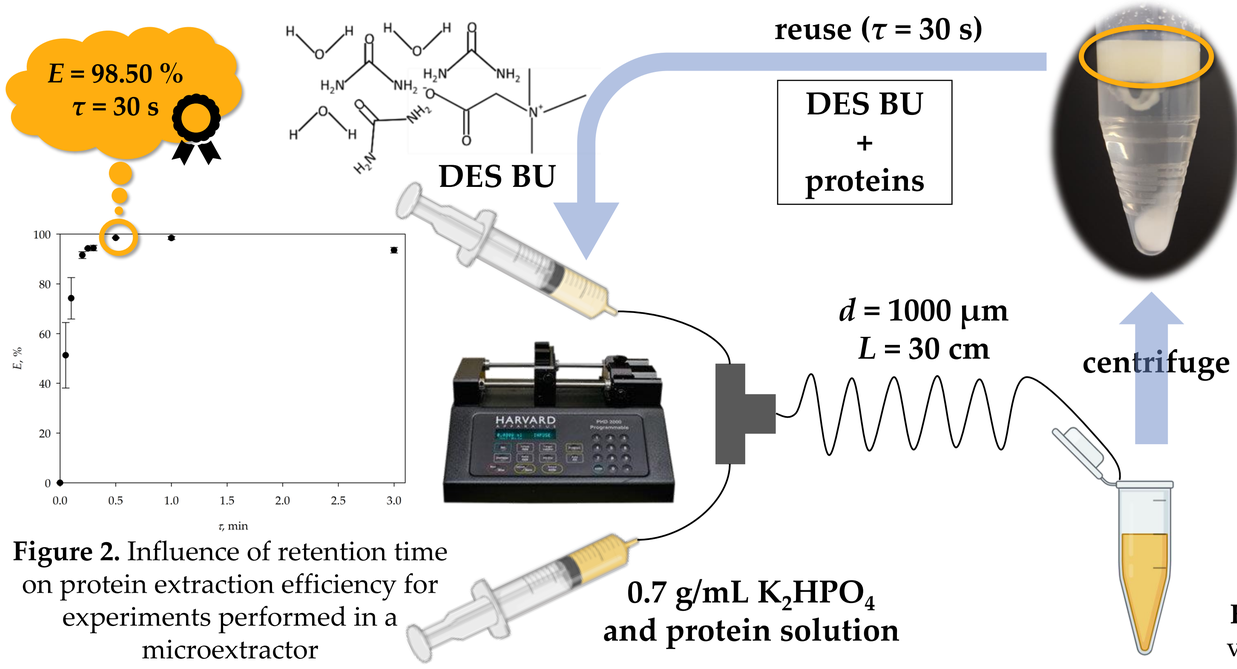


Figure 2. Influence of retention time on protein extraction efficiency for experiments performed in a microextractor

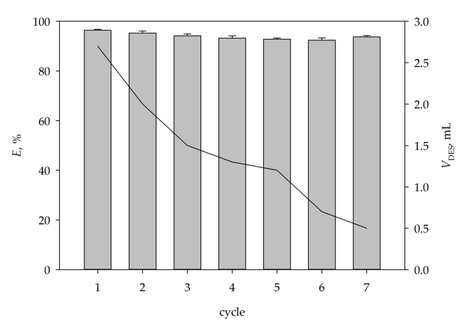


Figure 3. Extraction efficiency and DES volume after each microextraction cycle

✓ verification → raw lipase sample

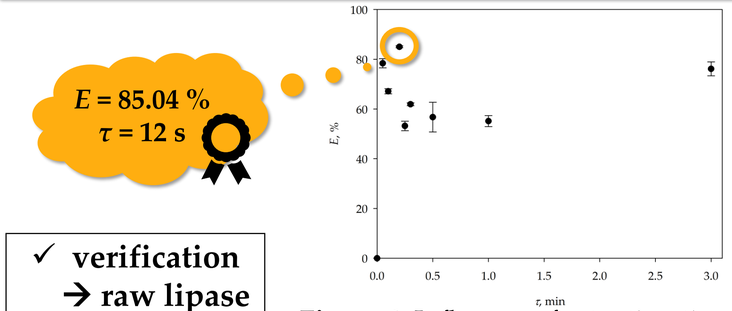


Figure 4. Influence of retention time on extraction efficiency for experiments performed with raw lipase in a microextractor

Conclusions

Aqueous two-phase systems based on six different natural DESs (Table 1.) for protein extraction were investigated. Optimal two-phase system features were determined through batch protein extraction experiments where the influence of salt concentration on protein extraction efficiency was monitored (Figure 1.). The highest efficiency of 94.70 % was achieved in ATPS based on DES BU with $\gamma_{K_2HPO_4}$ = 0.7 g/mL, so those conditions were declared optimal and used in further research. Protein extraction process was then transferred to a microextractor and carried out under optimal conditions at various retention times (Figure 2.). Highest extraction efficiency of 98.50 % was obtained for only 30 s which is an indication that the process was significantly intensified in comparison to batch experiments. Retention time of 30 s was therefore used in reusability experiments where DES was succesfully used in 7 cycles with efficiencies above 90 % (Figure 3.). Number of cycles could probably be even higher, but due to constant DES volume loss, additional cycles could not be conducted in this case. Finally, the developed extraction method was verified using raw lipase produced by *Thermomyces lanuginosus* solid-state cultivation on hull-less pumpkin oil pomace (Figure 4.) with somewhat lower efficiencies. However, considering that the highest protein extraction efficiency obtained with raw lipase sample was still relatively high (E = 85.04 % for retention time of only 12 s), this method can be considered as suitable for continuous purification of raw samples.

Literature: Miličević, N., Panić, M., Valinger, D., Cvjetko Bubalo, M., Benković, M., Jurina, T., Gajdoš Kljusurić, J., Radojčić Redovniković, I., Jurinjak Tušek, A., Development of continuously operated aqueous two-phase microextraction process using natural deep eutectic solvents, Sep. Purif. Technol., 244 (2020) 116746.